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1) Benecke et al. Antibodies raised against synthetic peptides react with choline acetyltransferase in various immunoassays and in immunohistochemistry. J. Neurochemistry. (1993) Vol. 61, No. 3, pp. 804-811.

2) Tuaillon et al. A lipoyl synthetic octadecapeptide of dihydrolipoamide acetyltransferase in primary biliary cirrhosis. J. Immunology. (1992) Vol. 148, No. 2, pp. 445-450.

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Antibodies Raised Against Synthetic Peptides React with Choline Acetyltransferase in Various Immunoassays and in Immunohistochemistry

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Abstract: Antisera were raised in rabbits against five synthetic peptides. These peptides have been identified as pot ntially antigenic epitopes from the sequence of porcine choline acetyltransferase (ChAT) using primary and secondary structure analysis. All five antisera recognized immunoaffinity-purified antigen from porcine brain in an ELISA and on western blots. Four antisera recognized ChAT on dot blots, and another four antisera reacted with native and degraded enzyme in a sandwich ELISA using monoclonal antibodies as the capture antibody. One peptide antiserum was of similar avidity in this sandwich ELISA as a polyclonal antibody raised against immunoaffinity-purified ChAT. The same antiserum reacted with the enzyme from human placenta in an ELISA and on western and dot blots and recognized ChAT in rat, primate, and human neurons. Thus, a single peptide (amino acids 168–189) provides the means for easy, reliable, and reproducible generation of antibodies against ChAT suitable for replacing conventional polyclonal and monoclonal antibodies. **Key Words:** Choline acetyltransferase—Antibodies—Peptides—Porcine brain—Human placenta—Human brain.
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Purification of choline acetyltransferase (ChAT) has been extremely difficult owing to the miniscule amounts of the enzyme present in nervous tissue of vertebrates (Rossier, 1981). By use of the hybridoma technology several groups succeeded in generating monoclonal antibodies against partially purified ChAT from various species (Wainer et al., 1984). A few authors subsequently used these monoclonal antibodies in immunoaffinity chromatography to obtain highly purified enzyme (Bruce et al., 1985; Johnson and Epstein, 1986; Braun et al., 1987; Ishida et al., 1987; Ostermann et al., 1990). In return, such preparations were then successfully used for the production of polyclonal ChAT antibodies.

To establish an alternative method independent of tedious ChAT purification procedures, we studied the production of ChAT antibodies by immunization with synthetic peptides. Five peptides were identified as potentially antigenic linear epitopes on porcine ChAT. We analyzed the ChAT amino acid sequence published by Berrard et al. (1987) using 12 structural parameters (Westarp et al., 1990), including surface antigenicity (compare Walter, 1986), internal repetitions, protease cleavage side clusters, and superposing gliding averages for the complete protein (Westarp et al., 1990). Five peptides from 13 to 23 amino acids long were defined for antibody production. Four of these peptides exhibit homologies from four to 19 amino acids with corresponding sequences of human placental ChAT published by Hersh et al. (1988) and Cervini et al. (1991).

The antisera produced in rabbits against these peptides were analyzed in different immunoassays to study their reactivity to native and denatured ChAT from porcine brain or human placenta. We evaluated their usefulness in immunohistochemistry of the vertebrate and human CNS.

EXPERIMENTAL PROCEDURES

Peptide synthesis

Peptides were synthesized using Fmoc (9-fluorenylmethoxycarbonyl)-protected, Py BOP (benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate)-activated amino acids (Coste et al., 1990) and an automatic peptide synthesizer (model 9050; Milligen, Eschborn, F.R.G.). After cleavage from the resin and the protecting groups, peptides were purified by reversed-phase chroma-

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Abbreviations used: BSA, bovine serum albumin; ChAT, choline acetyltransferase; KLH, keyhole limpet hemocyanin; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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tography using a Delta Pak C18 column (Millipore, Eschborn) and an elution gradient (50 min) from 0 to 50% acetonitrile containing 0.1% trifluoroacetic acid. The purity and the correct amino acid sequence were confirmed by HPLC, UV spectroscopy, mass spectroscopy, and sequence analysis.

Immunizations

The peptides, 10 mg each, were coupled to 20 mg of keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA), using the glutaraldehyde method of Messner et al. (1989). Antibodies against the peptides were produced by immunizing New Zealand rabbits (using two animals for each peptide) with 300 μg of KLH-coupled peptide in Freund's complete adjuvant followed by three boosts in 4-week intervals with half the amount of coupled peptide in Freund's incomplete adjuvant. Titers were monitored by ELISA using BSA-coupled peptide as the antigen. Three weeks after the last boost the animals were bled, and serum was prepared. Serum was frozen in aliquots at −30°C.

Purification of ChAT

Immunoaffinity purification of ChAT from porcine brain was carried out according to the method of Ostermann et al. (1990). Partial purification of ChAT from porcine brain and human placenta was achieved as described by Sabelhaus et al. (1990).

ELISA and sandwich ELISA

ELISA and sandwich ELISA measurements were carried out according to the technique of Ostermann-Latif et al. (1993) with the following modifications: Immunoaffinity-purified porcine brain ChAT was coated in a concentration of 1 mg/L, whereas partially purified human placental ChAT was used at 10 mg/L. The peptides were coated in a concentration of 0.1 mg/L, and BSA-coupled peptides were used at 1 mg/L. KLH and human IgG were coated in concentrations of 1 mg/L. The peptide antisera were used in a standard dilution of 1:4,000 and in various higher dilutions. A polyclonal antibody (19396) raised against immunoaffinity-purified ChAT (Ostermann et al., 1990) was used in a standard dilution of 1:3,000 and in various higher dilutions. In a sandwich ELISA, monoclonal antibodies [B3.9B3 and A10.29B4 (Ostermann-Latif et al., 1992)] were coated in concentrations of 5 mg/L. Protein A-purified and immunoaffinity-purified P3 antibodies were coated in concentrations of 5 mg/L. As detecting antibodies, the polyclonal ChAT antiserum (19396) was used in the same dilution as described above for the ELISA, P3 antiserum was used in a dilution of 1:2,000, and monoclonal antibody B3.9B3 was used at 5 mg/L. Immunoaffinity-purified ChAT from porcine brain was used as the antigen in a standard concentration of 1 mg/L and in various dilutions.

Western blots

Western blots were carried out as described by Ostermann et al. (1990). Three micrograms per lane of immunoaffinity-purified ChAT from porcine brain and 20 μg per lane of partially purified ChAT from human placenta were loaded on the sodium dodecyl sulfate (SDS)-polyacrylamide gels. Immunodetection was achieved using standard peroxidase protocols.

Dot blots

Four microliters of the following solutions was applied on nitrocellulose sheets (Schleicher and Schüll, Dassel, F.R.G.) using an Eppendorf micropipette: immunoaffinity-purified ChAT from porcine brain, 1 mg/L; partially purified ChAT from human placenta, 90 mg/L; peptides, 5 g/L; BSA-coupled peptides, 20 mg/L; and human IgG, 1 mg/L. Standard peroxidase protocols were used for immunostaining as described by Ostermann-Latif et al. (1990).

Immunoaffinity chromatography

KLH and peptide P3, respectively, were coupled to CNBr-activated Sepharose (Pharmacia, Freiburg, F.R.G.), and columns (1 × 5 cm) were prepared subsequently. The P3 antiserum was first passed through the KLH column. The effluent, containing mainly P3 antibodies and contaminating KLH antibodies, was then loaded onto the P3-conjugated Sepharose. The effluent of this column contained the remaining KLH antibodies, yet no P3 antibodies. This second column was subsequently eluted using 3.5 *M* $MgCl_2$. The eluate dialyzed immediately contained the P3 antibodies. The purification procedure was monitored by ELISA using KLH or P3, respectively, adsorbed to the microtiter plates.

Immunohistochemistry

Coronal sections through the human forebrain (n = 2), containing basal ganglia, septum, and nucleus basalis of Meynert, were prepared postmortem (at most 48 h). The tissue blocks were fixed in a 1:2 solution of 4% paraformaldehyde and 10% formalin in 0.1 *M* phosphate buffer (pH 7.4) at 4°C for 1 week. After fixation, the tissue was placed into increasing concentrations of sucrose (10–30% in phosphate buffer, pH 7.4) until equilibration. Frontal sections were cut frozen at a thickness of 50–60 μm, placed in a cryoprotective solution (30% sucrose and 30% ethylene glycol in 0.1 *M* phosphate buffer, pH 7.4), and stored at −15°C.

In addition, frozen cut frontal sections from brains of two rats (Sprague–Dawley; SAVO, Kissleg i. Allgäu, F.R.G.) and one marmoset (*Callithrix*) that had been fixed under deep anesthesia [pentobarbital sodium (Nembutal); 50 mg/kg] by transcardial perfusion with phosphate-buffered 4% paraformaldehyde and 0.1% glutaraldehyde (rats) or Zamboni's fixative (primate) were used for immunostaining. Selected sections were processed for immunohistochemistry as well as routine Nissl staining with cresyl violet. The basic procedures for immunohistochemistry have been described for ChAT staining by Ostermann-Latif et al. (1992).

RESULTS

Peptides, immunizations, and titers of antisera

Five peptides, designated P1–P5, were identified as potentially antigenic epitopes from the sequence of porcine ChAT by primary and secondary structure analysis (Westarp et al., 1990). The amino acid sequences of these peptides are given in Table 1. Peptides P1, P2, P3, and P4 share sequence homologies with the human placental enzyme. For determination of antibody titers, BSA-conjugated or free peptides were adsorbed to the polystyrene surface of microtiter plates. The titers measured ranged from 10^5 to 10^6 for BSA-conjugated peptides and were ~10^5 for free peptides (Table 2). Detection limits were 60–150 ng/L for BSA-conjugated and 70 ng/L–1.5 μg/L for free peptides (Table 2). The highest titers, ranging from 10^6 to

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TABLE 1. *Five possibly antigenic peptides as deduced from the sequence of porcine brain ChAT by primary and secondary structure analysis*

Peptide	Sequence	Amino acids	No. of amino acids
P1	[SL]SQSGMG[KPLATKE]	616–630	15
P2	LDAPGGMELSDTNR[ALQL]	283–300	18
P3	[GLFSSYRLPGHTQDTLVAQ]KSS	168–189	22
P4	[ESASI]RRFHEGRVDNIR[SATPEA]	448–471	23
P5	LLASSAEKLQQIV	387–399	13

The brackets indicate homologies with the human placental enzyme.

10^7, were obtained when uncoupled KLH was coated to the wells of microtiter plates. When BSA-conjugated peptides were reacted with the antisera, various cross-reactions with the other peptides were observed. Cross-reactions did not occur when uncoupled peptides were directly adsorbed to the immunoplate. Hence, this cross-reactivity may be due to antibodies directed against epitopes involving the glutaraldehyde bridge or some conformational similarities of the BSA-conjugated peptides (Harlow and Lane, 1988).

Reactions with ChAT from porcine brain

When immunoaffinity-purified ChAT from porcine brain was adsorbed to the surface of the microtiter plate, all five antisera raised against the KLH-conjugated peptides reacted with this ChAT preparation. The titers reached 10^5 and were comparable to those with uncoupled peptides (Table 2). The detection limits were in the range of 3–125 μg/L. The reaction of the P5 antiserum was clearly weaker than the reactions of the other antisera.

SDS-denatured immunoaffinity-purified ChAT from porcine brain was recognized on western blots by all five peptide antisera (Fig. 1). On dot blots ChAT was recognized well by anti-P1, -P2, and -P3, less by anti-P4, and scarcely by anti-P5 (Fig. 2). The antigen had not been SDS-denatured in this case and was presumed to retain, more or less, its native conformation (Shields et al., 1991).

A sandwich ELISA highly specific for porcine brain ChAT has been developed by Ostermann-Latif et al. (1993). This ELISA uses two monoclonal antibodies (A10.29B4 and B3.9B3) separately or in combination as the capture antibody. A10.29B4 preferably binds to native ChAT and additionally recognizes degraded ChAT. In contrast, B3.9B3 only reacts with a particular degradation product. Detection of the immobilized antigen was achieved with a polyclonal antiserum that was raised in rabbits against immunoaffinity-purified ChAT (Ostermann et al., 1990). The five peptide antisera were investigated as detector antibodies in this sandwich ELISA to evaluate the possibility of replacing the polyclonal ChAT antiserum by a specific peptide antibody. The P3 antiserum reacted in the sandwich ELISA with native (Fig. 3A) and degraded (Fig. 3B) ChAT as sensitively as the polyclonal ChAT antibody. The other peptide antisera were less sensitive in this immunoassay, depending on the monoclonal antibody used as the capture antibody (Fig. 3).

After protein A purification of the peptide antisera,

TABLE 2. *Determination of titers and detection limits of the antisera raised against the KLH-coupled peptides P1–P5*

	Antigen					
Antibody	BSA–peptide	Peptide	Porcine ChAT	Human ChAT	Human IgG	KLH
Anti-P1						
Titer	1:1,000	1:128	1:128	1:128	0	1:2,500
Detection limit	0.15	1.5	15	70	0	0.7
Anti-P2						
Titer	1:500	1:500	1:500	1:256	1:128	1:5,000
Detection limit	0.06	0.7	3	70	7	0.7
Anti-P3						
Titer	1:500	1:256	1:128	1:128	0	1:1,000
Detection limit	0.06	0.7	30	70	0	0.7
Anti-P4						
Titer	1:1,000	1:1,000	1:128	1:64	1:128	1:5,000
Detection limit	0.06	0.07	30	150	60	0.7
Anti-P5						
Titer	1:2,500	1:128	1:32	1:64	0	1:5,000
Detection limit	0.06	6	125	300	0	0.7

In an ELISA various antigens were adsorbed to the surface of a microtiter plate and reacted with the antisera. Porcine ChAT was immunoaffinity-purified from brain. Human ChAT was partially purified from placenta. Titers are given as ×1:1,000, and detection limits are given as μg/L.

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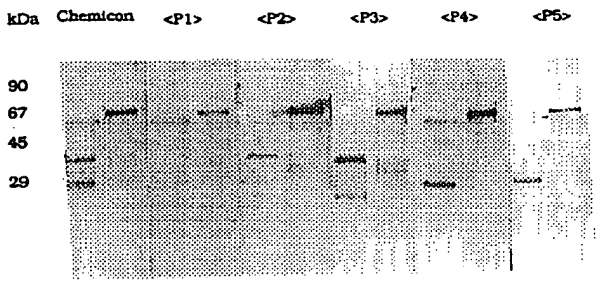

FIG. 1. Western blots of partially purified human placental ChAT (left lanes) and immunoaffinity-purified ChAT from porcine brain (right lanes) after SDS-PAGE. The antigens were immunodetected with a commercially available polyclonal anti-ChAT antibody raised against human placental ChAT (Chemicon) and with the peptide antibodies raised against peptides (P1–P5) deduced from the sequence of porcine brain ChAT. Immunostaining was achieved with a peroxidase-conjugated anti-rabbit IgG antibody.

their IgG fractions were adsorbed to the wells of the immunoplates to study the usefulness of these IgG as capture antibodies in a reversed sandwich ELISA that uses monoclonals as detector antibodies. Anti-P3 was the only antibody able to bind immunoaffinity-purified ChAT from porcine brain detectable with B3.9B3. A10.29B4 did not react with ChAT in this ELISA. Although this ELISA was less sensitive than the one described above (monoclonal antibodies as the capture antibody), its sensitivity could be remarkably improved after immunoaffinity purification of the P3 antibodies (see below and Fig. 4).

Reactions with human placental ChAT

The enzyme purified by immunoaffinity chromatography was not available for investigations with human placental ChAT. Instead, we used a partially purified preparation (Sabelhaus et al., 1990) with a specific activity of 2,500 U/mg of protein (1 U = 1 nmol of acetylcholine formed/min at 37°C); in comparison, the specific activity of immunoaffinity-purified ChAT from porcine brain was 12,500 U/mg of protein (Ostermann et al., 1990). A partially purified enzyme preparation of porcine brain purified by the same procedure as the placental enzyme and with a similar specific activity was, however, suitable for replacing immunoaffinity-purified ChAT in most experiments as described. Although the main portion of the partially purified ChAT preparation from porcine brain consisted of contaminating proteins, ChAT was well detected by monoclonal and polyclonal antibodies when this preparation was adsorbed to the wells of microtiter plates (Ostermann-Latif et al., 1992). When partially purified ChAT from human placenta was coated on the microtiter plates and reacted with the peptide antisera, titers were comparable to those with immunoaffinity-purified porcine ChAT. Lower titers were demonstrated for anti-P4 and anti-P5 than for the remaining three antisera (Table 2). The detection limits, however, were higher for human placental ChAT than for porcine brain ChAT and ranged from 70 to 300 μg/L. In contrast to immunoaffinity-purified ChAT from porcine brain, linearity was difficult to demonstrate with partially purified placental enzyme by antibody dilution in ELISA.

Anti-P2 and anti-P4 reacted with human IgG that was present in small amounts in the partially purified preparations from placental ChAT (Table 2).

In a sandwich ELISA human placental ChAT could not be studied because neither mono- nor polyclonal antibodies were available to immobilize the human enzyme.

All five antisera reacted with the partially purified human placental enzyme on dot blots. Cross-reactivity with human IgG was minimal in this assay (Fig. 2). After SDS-polyacrylamide gel electrophoresis (PAGE) of denatured partially purified human placental ChAT, diverse reactions were found with the peptide antisera on western blots (Fig. 1). It was difficult to immunostain the typical 68-kDa ChAT band.

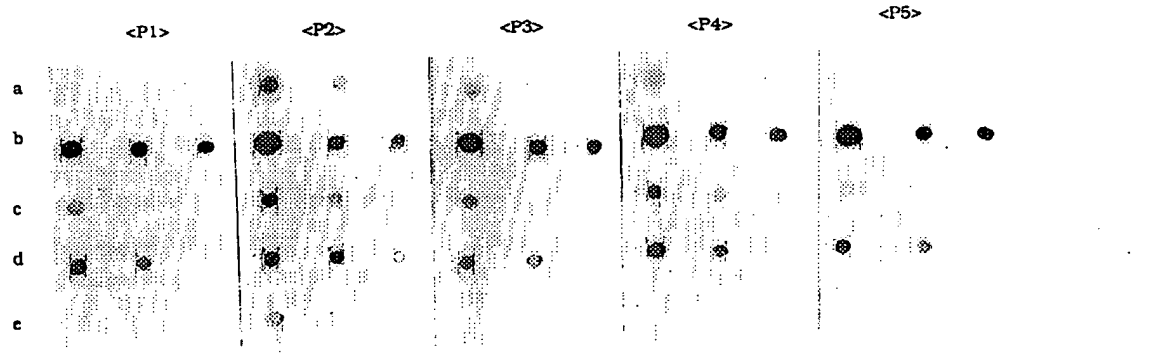

FIG. 2. Dot blot of various antigens on nitrocellulose immunodetected with peptide antibodies (compare Fig. 1). Four microliters of antigen was applied, with starting dilutions as follows (left lanes): a, peptides P1–P5 (5 mg/ml); b, BSA-conjugated peptides P1–P5 (20 μg/ml); c, immunoaffinity-purified ChAT from porcine brain (1 μg/ml); d, partially purified ChAT from human placenta (90 μg/ml); and e, human IgG (5 μg/ml). Middle and right lanes represent dilutions by a factor of 10 and 100, respectively. For immunostaining, standard peroxidase protocols were used (compare Fig. 1).

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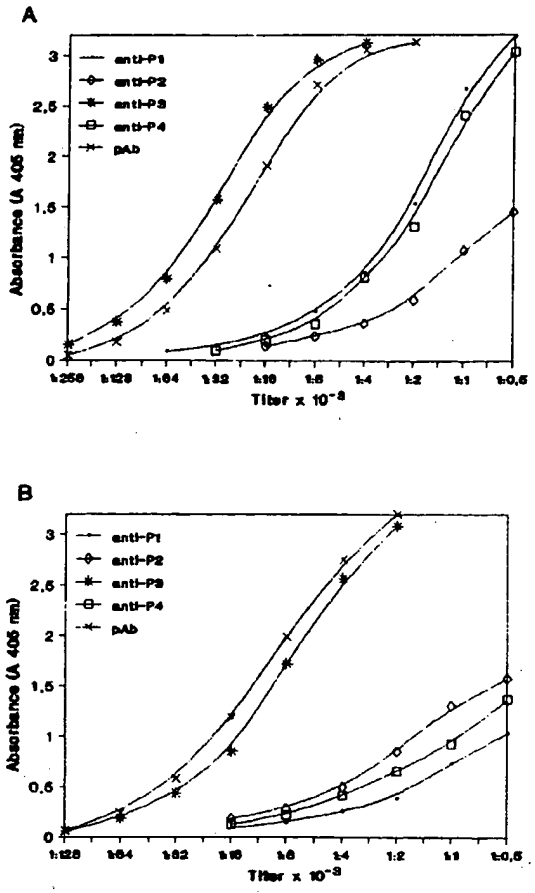

FIG. 3. Sandwich ELISA with monoclonal antibodies A10.29B4 (A) and B3.9B3 (B) as the capture and peptide antibodies (see Table 2) as the detector antibody. Immunoaffinity-purified ChAT from porcine brain was used as the antigen (1 μg/ml). Color reaction was achieved by use of a peroxidase-conjugated anti-rabbit IgG antibody. pAb, polyclonal antibody 19396 raised against immunoaffinity-purified ChAT from porcine brain.

This is demonstrated in Fig. 1 when the blot was immunostained with a commercially available polyclonal ChAT antibody (Chemicon; Paesel, Frankfurt, F.R.G.) raised against the human placental enzyme. A very faint reaction—two discrete bands, probably degradation products—could be observed with a molecular mass lower than that of immunoaffinity-purified porcine ChAT. We also observed two intensely stained bands at 45 and 30 kDa, which are presumed to be degradation products of ChAT, not uncommon in comparable preparations of porcine ChAT. Similar results were obtained with human placental ChAT immunostained with a high-titered polyclonal mouse antibody raised against immunoaffinity-purified porcine ChAT (authors' unpublished data). Our five peptide antisera bound to one or several of the assumed degradation products of ChAT (Fig. 1).

Immunoaffinity purification and characterizati n of P3 antibodies

The immunoaffinity-purified P3 antibodies were studied in the immunoassays described above and were found to yield similar or even better results (data not shown). When the purified P3 antibodies were adsorbed to the microtiter plates, the antigen was detectable with monoclonal antibody B3.9B3 but not with A10.29B4. Compared with the protein A-purified P3 antibodies the sensitivity of the ELISA could clearly be improved (Fig. 4). This was mainly due to the elevated concentration of specific P3 antibodies in this fraction. As evidenced by the radiometric assay of Fonnum (1975), the ChAT protein immobilized by P3 antibodies was inactive. The ELISA using immunoaffinity-purified P3 antibodies as the capture antibody was as sensitive as the reversed ELISA using the monoclonal B3.9B3 as the capture antibody and anti-P3 as the detector (Fig. 4).

Immunohistochemistry

In brain sections from the rat, monkey, and human, staining was best with antibody anti-P3. Anti-P4 resulted in weak immunostaining, whereas anti-P1, -P2, and -P5 were negative. Anti-P3 gave excellent results with negligible backround staining when used in dilutions of 1:500 or 1:1,000. Figures 5 and 6 depict several examples of ChAT-immunoreactive neurons and fibers in forebrain regions of the rat, nonhuman primate, and human. Regions containing the largest ChAT immunoreactivity included the septal nuclei, the nuclei of the diagonal band of Broca, the nucleus basalis of Meynert, and the caudate–putamen. Immunostaining was present in the cytoplasm and processes

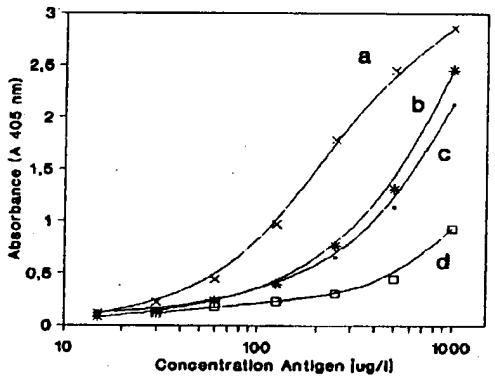

FIG. 4. Sandwich ELISA with monoclonal and polyclonal antibodies as capture and detector antibodies, respectively: curve a, monoclonal antibody B3.9B3 as capture and polyclonal antibody 19396 (pAb; see Fig. 3) as detector; curve b, B3.9B3 as capture antibody and P3 antibody as detector; curve c, immunoaffinity-purified P3 antibody as capture and B3.9B3 as detector; and curve d, protein A-purified anti-P3 IgG as capture and B3.9B3 as detector. Immunoaffinity-purified ChAT from porcine brain (1 μg/ml) was used as the antigen. Color reaction was achieved with standard peroxidase protocols.

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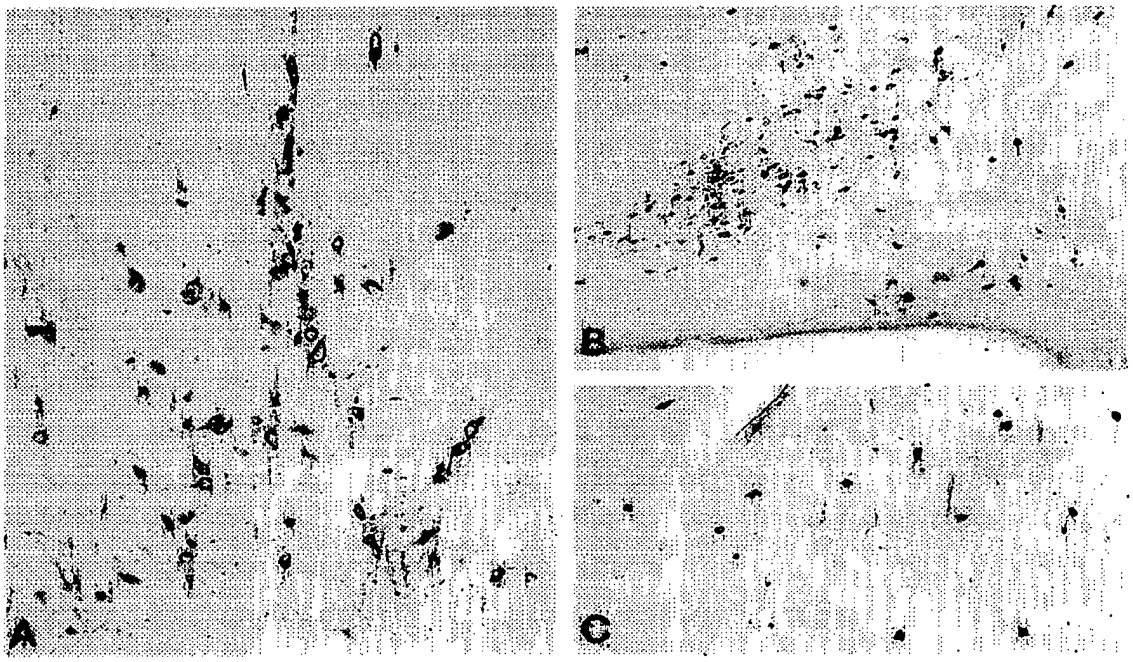

FIG. 5. ChAT-immunoreactive neurons in frontal sections through the rat (A) and marmoset (B and C) forebrain. A: Medial septum of the rat brain demonstrating a population of cholinergic neurons and fibers along the midline. B and C: In the prosencephalon of the marmoset numerous cholinergic cells are found in the nuclei of the diagonal band of Broca (B; horizontal limb) as well as throughout the caudate putamen (C). Magnification: A, ×126; B and C, ×50.

of cells. The morphological characteristics varied from large bi- and multipolar cells with long, branched processes found in the nucleus basalis of Meynert, to medium-sized multipolar cells, observed within the striatum. In addition, our findings indicate that several routine fixation procedures may be suitable for immunocytochemical detection of ChAT with anti-P3, i.e., immersion or perfusion with buffered formaldehyde or picric acid/formaldehyde solutions.

DISCUSSION

Antisera were raised in rabbits against five synthetic peptides deduced from the sequence of porcine brain ChAT (Westarp et al., 1990) to evaluate their antigenicity, which has been postulated by theoretical considerations (for a critical review, compare Walter, 1986). Because ChAT purification is rather inconvenient (Ostermann et al., 1990), it was desirable to find antisera for immunoassays without having to purify the enzyme. Our objective was to find peptide antibodies with defined specificity and a potential to replace polyclonal antisera or monoclonal antibodies in various immunoassays.

The antisera produced against five peptides all reacted with degraded antigen in ELISA and on western blots; titers and detection limits of the antisera differed. In a sandwich ELISA that used monoclonal antibodies as the capture antibody (Ostermann-Latif et al., 1993), one peptide antiserum (anti-P3) showed binding characteristics as the detector similar to those of a polyclonal antiserum raised against immunoaffinity-purified ChAT from porcine brain. Active enzyme immobilized by the monoclonal antibody A10.29B4 (Ostermann-Latif et al., 1993) was recognized in the sandwich ELISA by four peptide antisera with differing avidities and detection limits. Degraded enzyme immobilized by the monoclonal antibody B3.9B3 (Ostermann-Latif et al., 1993) was less well recognized. After immunoaffinity purification the antibodies against peptide P3 were useful as capture reagents in a reversed ELISA, and detection could be achieved with the monoclonal B3.9B3; this ELISA was as sensitive as the reversed ELISA using B3.9B3 as the capture antibody. In conclusion, antisera raised against five synthetic peptides reacted with degraded and native porcine antigen, thus confirming the criteria applied to select the peptides (Westarp et al., 1990).

When the peptide antisera were reacted with human placental enzyme, our results were not as uniform as with porcine brain ChAT, probably because of a lack of immunoaffinity-purified antigen. Some of the antisera reacted with placental ChAT in ELISA, on dot blots, and on western blots. Sandwich ELISA reactivities could not be tested for the human enzyme

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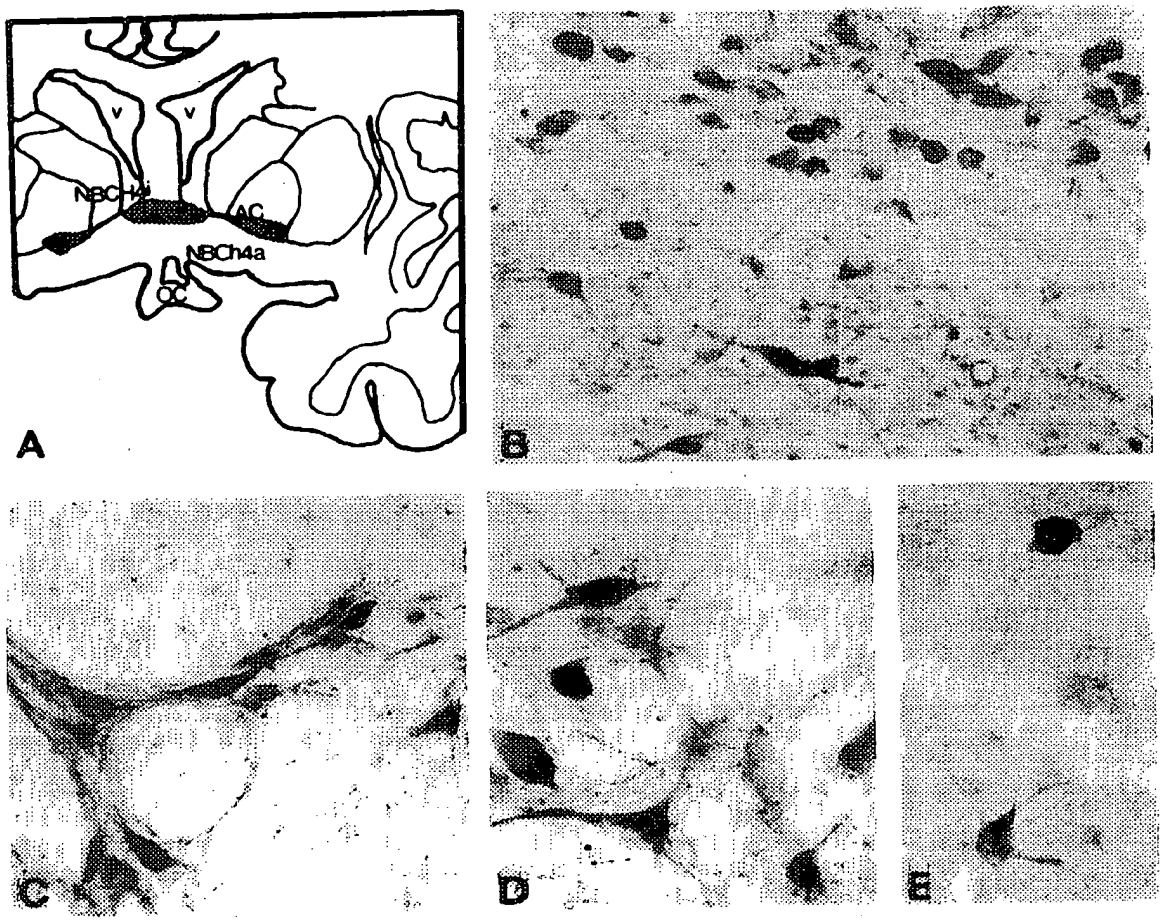

FIG. 6. Frontal section through the human forebrain. **A:** drawing of the original slide indicates different subregions of the nucleus basalis of Meynert (NBM), depicted at higher magnification in **B–D.** V, ventricles; AC, anterior commissure; OC, optic chiasm; NBCh4a, anterior portion of the nucleus basalis. **B:** ChAT-immunoreactive branching neurons in the NBCh4a. C and D: A population of ChAT-containing cells localized along the globus pallidus in the NBCh4i region. E: ChAT-containing neurons scattered throughout the neostriatum. Magnification: A, ×0.9; B and C, ×198; D and E, ×315.

because monoclonal antibodies reactive with human ChAT were not available. The reaction of P3 antibodies with human placental ChAT was superior to that of polyclonal porcine ChAT or of other peptide antibodies. This was not surprising as the sequence of this peptide shares the highest degree of homology (83%) with its corresponding human sequence.

The distribution and morphology of ChAT-immunoreactive neuronal structures found with P3 antibodies in the brains of various mammalian species, including humans, are in good accordance with previous investigations using poly- (German et al., 1985) and monoclonal (Ostermann-Latif et al., 1992) antibodies against ChAT.

Peptide 168–189 (P3) from porcine ChAT or its consensus sequence from human placental ChAT, amino acids 168–185 (Table 1), thus promises a reproducible, reliable, and relatively easy access to defined and useful ChAT antibodies needed for animal and human immunohistochemistry and other immunoassays.

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